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14. ABSTRACT (200 Words) Summary of most significant finding (s) <u>Purpose:</u> to develop and validate objective tests to diagnose vision deficits in patients with cognitive impairment and ensure effective monitoring of their treatment. <u>Scope:</u> objective methods to monitor visual function include 1) the pupil light reflex (PLR), 2) light evoked potentials (VEP) from the brain and from the eye and 3) purposeful eye movements to track moving targets that are resolved. <u>Major Findings (year 4):</u> i) determined that the melanopsin mediated pupil response, derived from the differential sustained pupil response to blue light vs. red light, correlates highly with retinal ganglion cell layer thickness derived from optical coherence tomography (OCT) in eyes with optic neuropathy, ii) in terms of the red-blue pupil light reflex test, showed that the resulting responses measured with our custom DynaScan-Smart Eye system correlate well with results obtained with the clinically-used Neuroptics DP-2000 system, iii) found that a patient has greater difficulty to stay fixated on a fixation target during stimulation of a diseased eye. <u>Significance:</u> objective tests of vision will greatly improve eye care by providing faster, lower cost testing that can be performed in remote settings and will provide a new tool for assessing innovative treatments being developed to save or restore vision.				
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## INTRODUCTION

Our project's research goal is to provide an objective and military relevant means for diagnosing and localizing the site of visual dysfunction in cognitively impaired patients. The successful attainment of this goal hinges on the development of a suite of objective tests designed to free the TBI patients from the cognitive demands placed on them during standard visual testing. As a necessary step for testing of cognitively impaired patients with suspected visual dysfunction, we will first optimize three objective tests of visual function: a) pupil contractions to light stimuli, b) evoked potentials elicited from the visual cortex in response to visual stimuli in the central and peripheral visual field locations, and c) eye position correlated with moving visual targets varying in spatial properties as a means of verifying that the patient was able to perceive the targets. Next, we will validate these objective tests against gold standard behavioral tests of visual field sensitivity in cognitively intact participants who are capable of performing these tests accurately. For the validation phase, participants will be selected who have either normal visual function or who have known dysfunction at different sites along their visual pathway. Using this strategy, normal eyes and eyes with well-defined damage to the retina, optic nerve, visual radiations or visual cortex will be used to study the sensitivity and specificity of the different modalities of objective testing being evaluated in this proposal. Once validated in these participants, these objective tests can then be rapidly implemented for use in cognitively impaired patients, specifically those who have suffered traumatic brain injury.

## **BODY**

### **RESEARCH ACCOMPLISHMENTS ASSOCIATED WITH APPROVED STATEMENT OF WORK FOR YEAR 4:**

#### **Task 1. Implementation of novel product-ready hardware solutions that allow objective testing of the visual system**

##### **1a. Hand-held portable pupillometer (Neuroptics, Inc.)**

We have developed unique, rapid testing protocols for assessing afferent visual function using the pupil light reflex. Collaborating with Neuroptics Inc (Irvine, CA), a low cost binocular portable battery powered pupillometer has been developed for commercial sale and distribution ("RAPiDo"). The prototype portable instrument will be tested in the next year. Its unique design allows it to reside on a desktop for outpatient testing or after removal from its support, it can be hand held to test inpatients, including those in the supine, lying flat position (Figure 1). This will be particularly useful for pupil testing of patients near battlefield arenas at triage points. We worked with Neuroptics on the design of this portable pupillometer and have specified that either the right eye or left eye can be stimulated independently or both eyes simultaneously, while both the right and left pupils are recorded simultaneously. This will allow assessment of both afferent and efferent defects in patients.

Our contribution to this commercial device has been the development of rapid pupil testing protocols for assessing the relative afferent pupil defect (log unit RAPD) that only takes 30 seconds of testing. During the last quarter, we have reported on the use and test results of the continuous RAPD test protocol (log unit RAPD as a function of stimulus light intensity) at the Association for Research in Vision and Ophthalmology (ARVO) meeting (May, 2014, Orlando, FL). Neuroptics Inc. is very interested in licensing the test protocol from us (the University of Iowa Research Foundation), and steps are underway to include it as part of their new RAPiDo device. In addition, we are developing novel, short testing protocols to assess the status of the retina and optic nerve in each eye independent of the other eye, using red and blue light stimuli (as discussed later in the report). This will allow a quantification of retina and optic nerve function in patients that have bilateral damage.

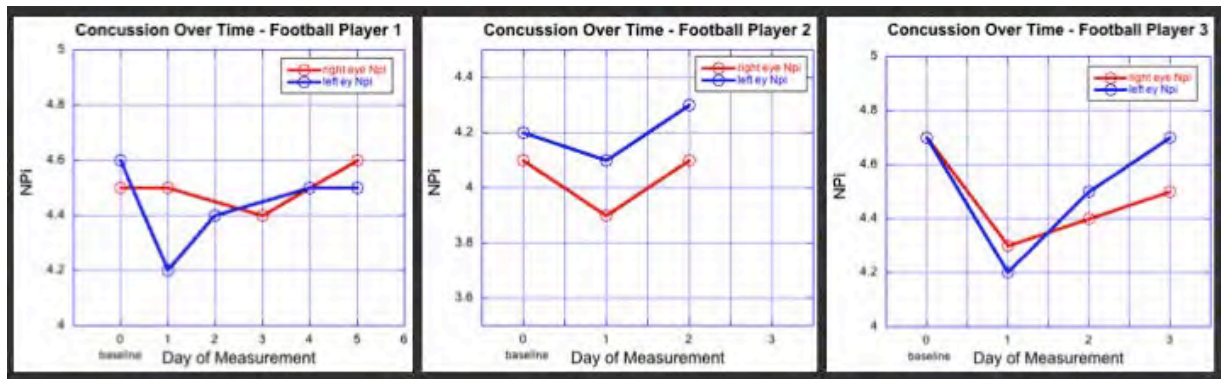


**Figure 1. Prototype binocular portable and desktop infrared computerized pupillometer (Neuroptics Inc, Irvine, CA) for fast, efficient clinical testing of the pupil light reflex. The pupillometer is battery powered and transmits video and pupil information by blue tooth to the portable processor or also to a nearby computer. The optical head can be easily removed and mounted on a handle for testing of patients that cannot sit up at a desktop area and for inpatients in a hospital bed.**

Another important finding that we have made during the past year is that the dynamics of the pupil light reflex, distilled into one response parameter, termed the Neurological Pupil Index (NPi), appears to correlate with severity of traumatic brain injury (TBI). The NPi may be a very useful, objective indicator of acute TBI. We have found that in the setting of the Emergency Room, the NPi, recorded in the trauma bays where the patients were being evaluated, predicted outcome measures. The outcome measures that were significantly related to the NPi were Glasgow Coma Scale, need for admission to the hospital and duration of hospitalization, presence of abnormal head CT scan, need for neurosurgical intervention, and discharge status (home vs. care center).

During the first quarter, we also completed our first study of the NPi in the University of Iowa Football Team. We measured the NPi in all players at preseason training camp and post-season. During the season, we also re-measured any players that were taken out of play during games for concussion evaluation. During the season, 3 players were taken out of play and evaluated with cognitive, neurological and balance testing each day until they were deemed fit for play. We measured the NPi in these 3 players on each day and compared the NPi (scale of 0-5; with 2SD of normal being from 3-5). Remarkably, in all 3 players, their NPi value dropped from their pre-season baseline value after the concussion and then returned to baseline by the time they returned to play (Figure 2), further

indicating that the NPi may be a valuable biological marker of TBI and would have military applications.



**Figure 2. Neurological Pupil Index (NPi) is shown (y axis) in 3 different Iowa Football Team players being evaluated daily for concussion. The right eye NPi is shown in red and the left eye in blue. The time scale on the x-axis is in days. Note that in all 3 players the NPi decreased from pre-season baseline value and then returned to baseline at the day when they were considered fit for return to play, based on their neurological exam, balance testing, and neurocognitive testing.**

#### 1b. Pupil testing protocol and advanced analysis

During the past year, we have tested additional patients using our new 30 seconds protocol for automatically determining the log unit relative afferent pupil defect (RAPD). The RAPD is an important objective measure of asymmetry of retina or optic nerve input between the two eyes due to disease. We found a very high correlation ( $R > 0.9$ ) between the machine automated determination of the RAPD and the clinical log unit RAPD determination made using neutral density filters with an alternating “swinging flashlight” test. We also found a significant correlation between the automated log unit RAPD and the asymmetry of the inner retinal layer thickness (retinal ganglion cell layer), as determined by optical coherence tomography (OCT) imaging in a subset of the same patients who had permanent structural loss of retinal neurons. Furthermore, we found that the automated log unit RAPD also can be affected by how bright the alternating light level is during the test, which may be specific for different types of damage to the retina and optic nerve. We plan to develop this idea further by reporting the RAPD as a function of how bright the stimulus light is for each patient and this is envisioned to provide an incremental increase in diagnostic information.

Based on the encouraging results from the continuous RAPD testing and analysis protocol developed as part of this project, we have submitted an Invention Disclosure to the University of Iowa Research Foundation, titled "Objective Methods of Testing Visual Function Using the Pupil Light Reflex", with an eye on patenting the concept. Here follows an excerpt of the disclosure:

"We describe a method to estimate the degree of asymmetry in signal transmission between the two eyes, which is based on testing the eyes with a sequence of light stimuli that alternates between the eyes, while measuring changes in pupil size of each eye over time due to the pupil light reflex (PLR). The method takes as input, data from the stimulus sequence presented to the subject or patient, as well as pupil size measurements, and employs curve fitting to estimate the asymmetry (RAPD = relative afferent pupillary defect). (Both afferent and efferent RAPD). To estimate the pure afferent component, the right and left pupil dynamics are recorded simultaneously in response to each light stimulus and averaged together for the right eye stimulus and then for the subsequent left eye stimulus. By averaging the right and left pupil responses together, and differences due to efferent wiring to the pupil are nullified and only the afferent difference between the right eye's input compared to the left eye's input is derived. Conversely, by averaging the right pupil response to a right and left eye stimulus and comparing it to the left pupil response to the same stimuli, we can isolate the efferent component of asymmetry between the output to the right and left pupils. This would give information about disorders that differentially affect the dynamics of pupil contraction and dilation from diseases affecting the output pathways controlling pupil size originating in the brain and passing to the iris of each eye. An example would be a third nerve palsy with pupil fiber involvement of the parasympathetic nerves to the iris sphincter muscles, such as what can occur with a cerebral aneurysm, or a Horner's syndrome from disorders that affect the sympathetic nerves to the iris dilator muscles. To our knowledge, no one has ever been able to quantify the asymmetry of efferent involvement in log units, similar to what has been done with quantifying the afferent asymmetry in log units.

The application would be highly useful in objectively screening patients for disorders affecting the retina, optic nerve or the efferent nerve pathways supplying the iris muscles as well as disorders affecting the iris muscles directly. Besides ophthalmology, neurology and neurosurgery, the application would also benefit primary and emergency care where a physician or physician extender may not have the skills or knowledge to be able to assess the visual system. Another important application would be its use in home care devices that could measure the aforementioned functions over time to understand if there is a worsening or improvement and response to treatment."

As part of a separate study, we have recently determined that the melanopsin mediated pupil response, derived from the differential sustained pupil response to blue light vs. red light, correlates highly with retinal ganglion cell layer thickness derived from optical coherence tomography (OCT) in eyes with optic neuropathy due to multiple sclerosis. We hypothesize that it will pave the way for determination of afferent input of each eye independent of the other and will be especially useful in patients with bilateral disorders of visual function, in which there may not be a relative afferent pupil defect. Based on our



initial findings, we have submitted an Invention Disclosure to the University of Iowa Research Foundation, titled "Use of the Pupil Light Reflex to Chromatic Stimuli to Track Optic Neuropathy", with an eye on patenting the concept. Here follows an excerpt of the disclosure:

"We describe a method to objectively diagnose the presence and severity of optic neuropathy within an individual eye independently, without having to reference it to the other eye as an asymmetry index. Alternating a light stimulus between the right and left eyes and comparing responses between eyes only has diagnostic value in case of unilateral disorders or disorders that are asymmetric in how much they affect the right eye compared to the left eye. Asymmetry detection is clinically useful but is limited to comparison of the two eyes to one another. The method outlined here specifically makes it possible to use each eye as its own control, and minimizes the impact of central nervous system influences on measurements of visual function and pupil response that are unrelated to the condition affecting vision. Such central nervous system influences having to do with state of excitement or sleepiness can cause fluctuations in the response of the pupil unrelated to the eye's function and can confound the diagnosis of ocular disorders, which is a deficiency of existing approaches to use the pupil light reflex as a measure of ocular visual function independent of the other eye. The method outlined here is based on testing an eye with a sequence of red and blue light stimuli (but not limited to only these colored stimuli), while measuring the response in pupil size over time due to the pupil light reflex (PLR). The method takes as input, data from the stimulus sequence presented to the subject or patient, as well as pupil size measurements, and estimates the degree of functional loss caused by disorders of the photoreceptors (rods and cones) and differentiates it from functional loss caused by disorders of the retinal ganglion cells of the tested retina as can occur in disorders of the inner retina, optic nerve, chiasm, or optic tract."

1e. Multi-camera eye movement monitor (Smart Eye AB) and visual stimulus software platform


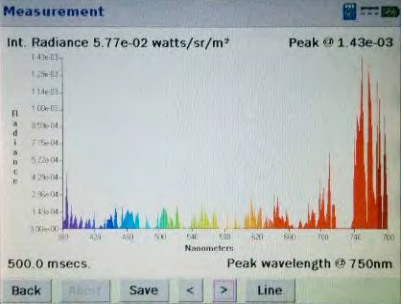

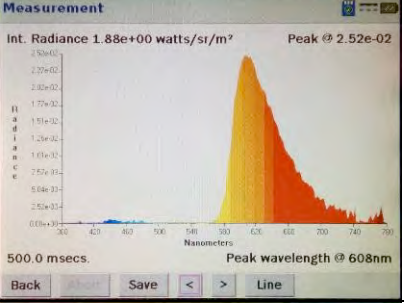

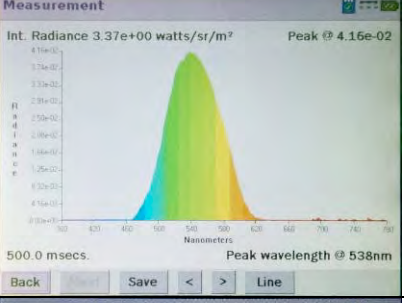

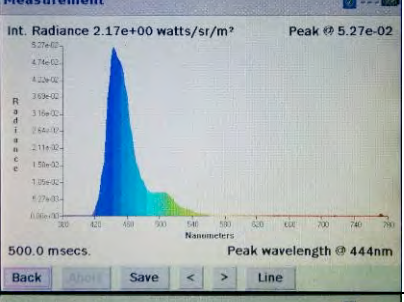
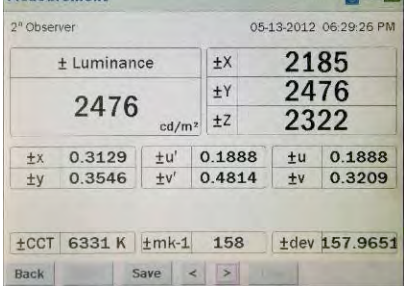
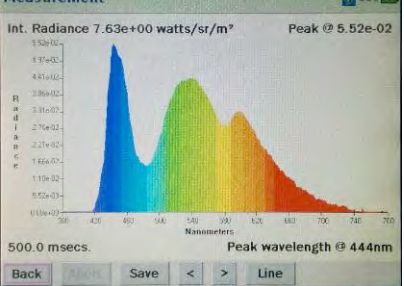
#### Visual stimulus presentation and data analysis software platform

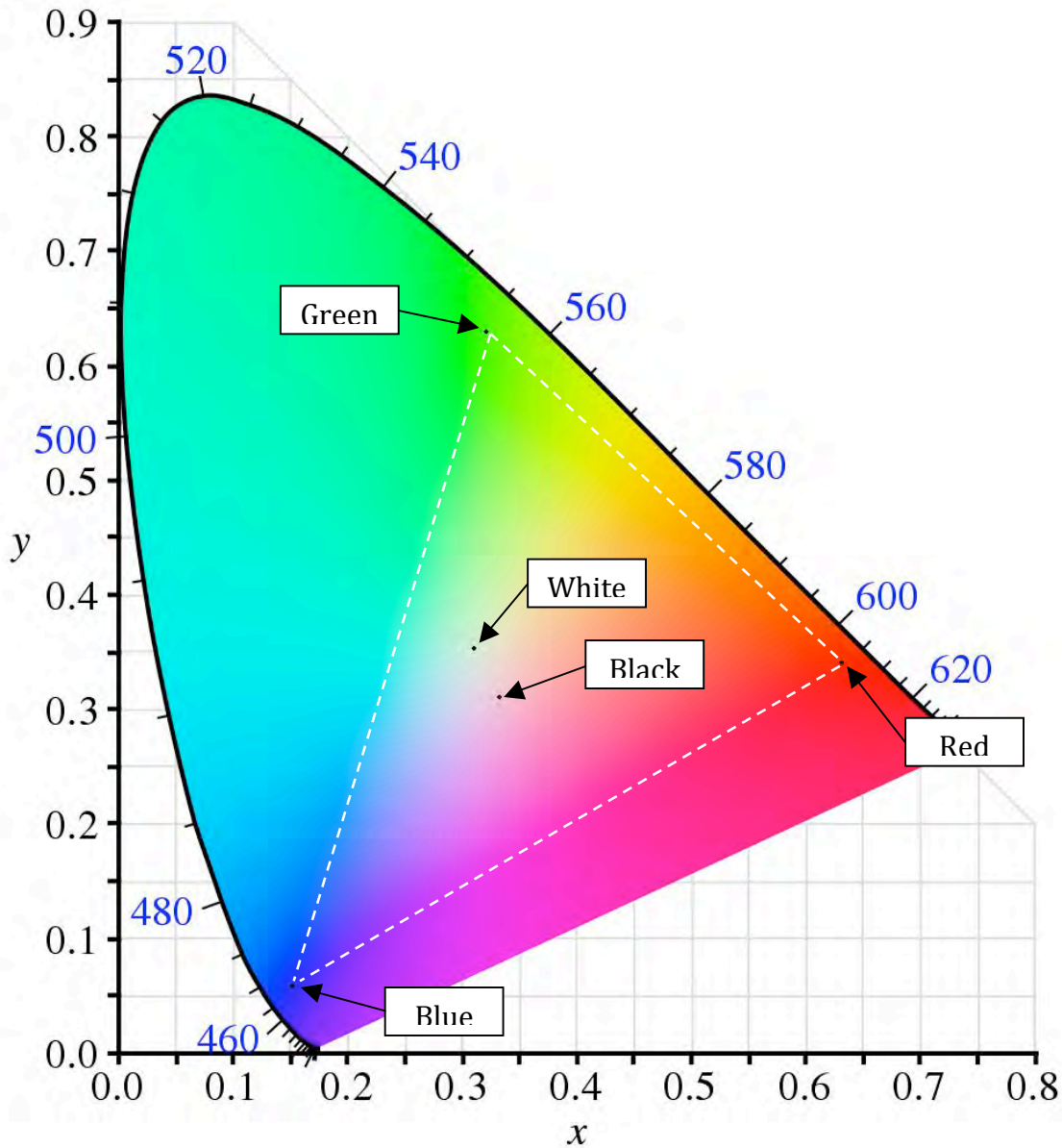
During the first quarter, we have collected calibration data for our super-bright DynaScan TV to enable us to accurately relate and compare ocular response measurements from different clinical stimulus presentation platforms -- such as the DP2000 desktop pupillometer and the Diagnosys Ganzfeld bowl stimulator-- with response measurements collected from our integrated TV-based system (DynaScan monitor). Dr. Chris Johnson assisted us in taking radiometric readings with 3 different types of light meters; a Photo Research PR-655 SpectraScan® Spectroradiometer (radiometric), and photopic light level readings with a PR-1980A Pritchard® Photometer and Extech Foot Candle Light Meter. The different readings are shown in Table 1 with illustrations and plotted in color space in Figure 3 below.

Apart from chromaticity values, we were also interested in calibration data for the range of brightness levels for the DynaScan TV, given that we have to turn off the light source of the TV -- by setting the brightness level to 0% -- during inter-stimulus intervals of pupil light

reflex experiments in order to maintain a sufficiently dark environment for light off conditions of testing. We have also updated the experiment control software to dynamically alter the brightness levels of the TV in addition to setting RGB values for optimizing the spectral distribution of both low and high intensity red and blue 1-second flashes that we are using to elicit pupil light reflexes.

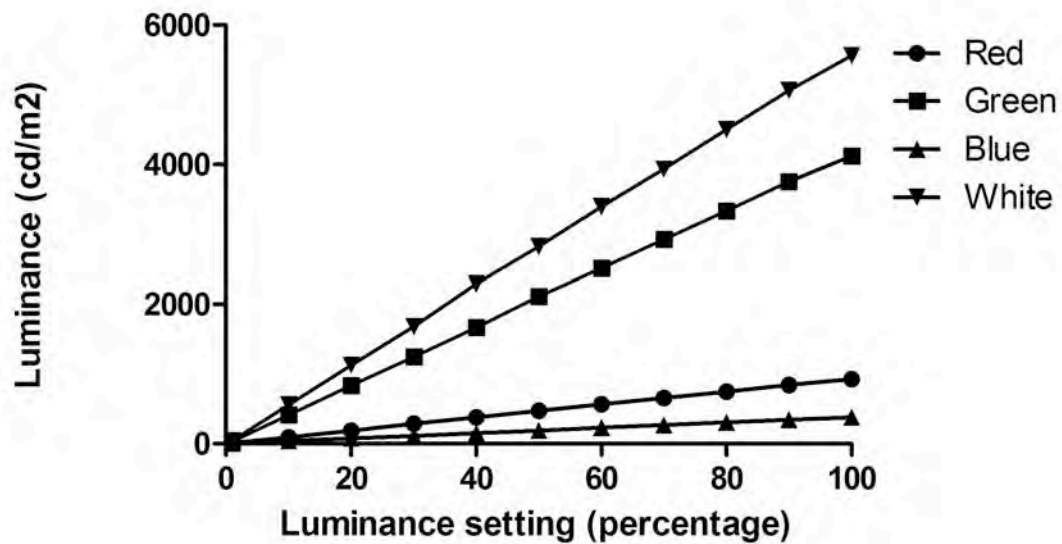
**Table 1. Radiometric data collected from DynaScan monitor using the Photo Research PR-655 SpectraScan® Spectroradiometer**

Color	Chromaticity Values	Spectral Distribution
<b>Black</b> <b>sRGB = [0;0;0]</b> <b>@ 50% brightness</b>		
<b>Red</b> <b>sRGB = [255;0;0]</b> <b>@ 50% brightness</b>		
<b>Green</b> <b>sRGB = [0;255;0]</b> <b>@ 50% brightness</b>		
<b>Blue</b> <b>sRGB = [0;0;255]</b> <b>@ 50% brightness</b>		
<b>White</b> <b>sRGB = [255;255;255]</b> <b>@ 50% brightness</b>		



**Figure 3. CIE chromaticity values for the DynaScan TV monitor which will be used for combined testing of the pupil light reflex, eye movements to visual stimuli and visual evoked responses from the eye and visual cortex.**

Figure 4 and Table 2 demonstrate that the light intensity response (luminance) of the DynaScan monitor is highly linear with the software luminance settings for each of the spectral stimuli (red, green, blue and white).



**Figure 4. Brightness level (luminance) settings (x-axis) and the corresponding photopic luminance readings recorded by the PR-1980A Pritchard® Photometer. The linear regression goodness-of-fit  $r^2$ -values are better than 0.999 for all colors.**

**Table 2. Illuminance readings in lux recorded with the Extech Foot Candle Light Meter at 7 feet in front of the DynaScan TV**

Brightness level	Red	Green	Blue	White
1%	-	6.5	-	7.5
2%	0.2	17.2	-	22.6
3%	5.4	24.8	1.1	32.3
5%	7.5	43.1	2.2	56.0
20%	37.7	183.0	10.8	236.8
50%	95.8	466.1	29.1	602.8
80%	152.8	742.7	46.3	963.4
100%	190.5	920.3	58.1	1197.0

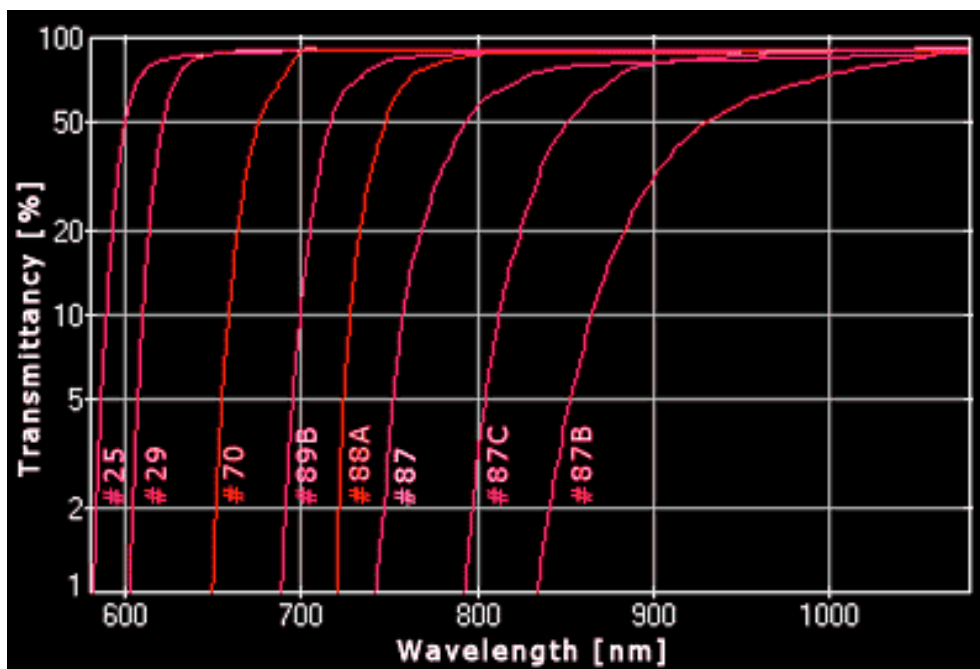
In order to measure a subject's monocular input for eliciting pupil light reflexes, eye movements and VEP responses, we have assembled two pairs of comfortable, disposable, over-sized ocular frames, commonly used as eye shields in the operating room. One pair is fitted with an infrared (IR) filter covering the left eye, and the other pair with an IR filter covering the right eye (Figure 5). The IR filters were cut from Kodak Wratten 87C sheets. The 87C filter attenuates slightly too much IR illumination of the covered eye while blocking visible light, resulting in suboptimal tracking of the covered pupil by the Smart Eye head and eye tracking system. We are currently optimizing this setup by finding the best wavelength spectra of a variety of IR filters to maximize the IR illumination of the covered eye while minimizing leakage of long wavelength red light from the TV through the filter (Figure 6). The goal is to occlude the visible light stimulus to either the right or left



eye while still being able to track eye and pupil movement by infrared light and our infrared sensitive video cameras.



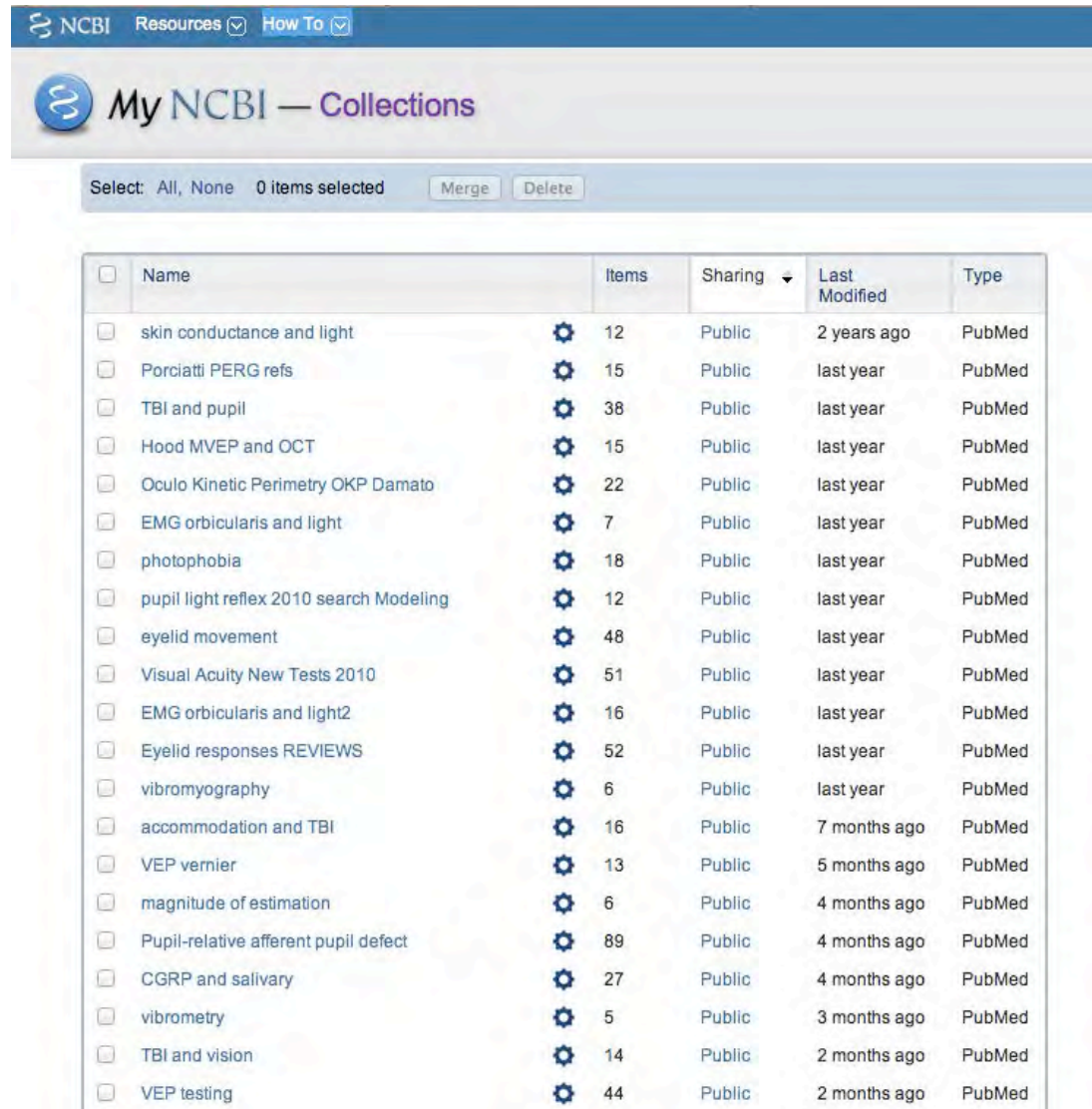
**Figure 5. IR filters fitted to two pairs of glasses for covering either the left (red pair) or the right eye (green pair) to block visible light from the DynaScan TV during monocular testing conditions, yet transmit infrared light emitted from directional infrared emitting diodes (LEDs). The other eye sees through a wide window cut in the clear plastic film.**



**Figure 6. Transmission spectral distributions of different Kodak IR filters. Currently, we are using type 87C, but we plan to try out types 87 and 88A in order to maximize the IR illumination of the covered eye needed by the Smart Eye head and eye tracking system to track the covered pupil. This will also minimizing transmission of visible long wavelength red light from stimuli provided by the TV.**

## 1e. Literature study

Completed in year 1 and reported in Year 1 annual report :



The screenshot shows the MyNCBI Collections interface. At the top, there is a navigation bar with 'NCBI', 'Resources', and 'How To' links. Below this is the 'MyNCBI — Collections' header. A status bar indicates 'Select: All, None 0 items selected' with 'Merge' and 'Delete' buttons. The main content is a table of search results.

<input type="checkbox"/>	Name		Items	Sharing	Last Modified	Type
<input type="checkbox"/>	skin conductance and light	⚙	12	Public	2 years ago	PubMed
<input type="checkbox"/>	Porciatti PERG refs	⚙	15	Public	last year	PubMed
<input type="checkbox"/>	TBI and pupil	⚙	38	Public	last year	PubMed
<input type="checkbox"/>	Hood MVEP and OCT	⚙	15	Public	last year	PubMed
<input type="checkbox"/>	Oculo Kinetic Perimetry OKP Damato	⚙	22	Public	last year	PubMed
<input type="checkbox"/>	EMG orbicularis and light	⚙	7	Public	last year	PubMed
<input type="checkbox"/>	photophobia	⚙	18	Public	last year	PubMed
<input type="checkbox"/>	pupil light reflex 2010 search Modeling	⚙	12	Public	last year	PubMed
<input type="checkbox"/>	eyelid movement	⚙	48	Public	last year	PubMed
<input type="checkbox"/>	Visual Acuity New Tests 2010	⚙	51	Public	last year	PubMed
<input type="checkbox"/>	EMG orbicularis and light2	⚙	16	Public	last year	PubMed
<input type="checkbox"/>	Eyelid responses REVIEWS	⚙	52	Public	last year	PubMed
<input type="checkbox"/>	vibromyography	⚙	6	Public	last year	PubMed
<input type="checkbox"/>	accommodation and TBI	⚙	16	Public	7 months ago	PubMed
<input type="checkbox"/>	VEP vernier	⚙	13	Public	5 months ago	PubMed
<input type="checkbox"/>	magnitude of estimation	⚙	6	Public	4 months ago	PubMed
<input type="checkbox"/>	Pupil-relative afferent pupil defect	⚙	89	Public	4 months ago	PubMed
<input type="checkbox"/>	CGRP and salivary	⚙	27	Public	4 months ago	PubMed
<input type="checkbox"/>	vibrometry	⚙	5	Public	3 months ago	PubMed
<input type="checkbox"/>	TBI and vision	⚙	14	Public	2 months ago	PubMed
<input type="checkbox"/>	VEP testing	⚙	44	Public	2 months ago	PubMed

**Figure 13. Example of literature search on topics in PubMed that was performed.**

The literature search is an ongoing process, however, as we continually are updating our information with recently published articles that are relevant to our proposed research. The literature search for papers focused on objective testing of visual function revealed the following relevant publications:

1: Ciocler Froiman P, Dantas PE. Assessment of visual acuity in patients with dementia using teller acuity cards. *Strabismus*. 2013 Jun;21(2):93-7. doi: 10.3109/09273972.2013.787634. PubMed PMID: 23713929.

2: Wood JS, Firbank MJ, Mosimann UP, Watson R, Barber R, Blamire AM, O'Brien JT. Testing visual perception in dementia with Lewy bodies and Alzheimer disease. *Am J Geriatr Psychiatry*. 2013 Jun;21(6):501-8. doi: 10.1016/j.jagp.2012.11.015. Epub 2013 Jan 11. PubMed PMID: 23567415.

3: Gundogan FC, Tas A, Altun S, Oz O, Erdem U, Sobaci G. Color vision versus pattern visual evoked potentials in the assessment of subclinical optic pathway involvement in multiple sclerosis. *Indian J Ophthalmol*. 2013 Mar;61(3):100-3. doi: 10.4103/0301-4738.99842. PubMed PMID: 23514643; PubMed Central PMCID: PMC3665036.

4: Bedell HE, Stevenson SB. Eye movement testing in clinical examination. *Vision Res*. 2013 Sep 20;90:32-7. doi: 10.1016/j.visres.2013.02.001. Epub 2013 Feb 15. PubMed PMID: 23416869.

5: Dong W, Yan B, Johnson BP, Millist L, Davis S, Fielding J, White OB. Ischaemic stroke: the ocular motor system as a sensitive marker for motor and cognitive recovery. *J Neurol Neurosurg Psychiatry*. 2013 Mar;84(3):337-41. doi: 10.1136/jnnp-2012-303926. Epub 2012 Dec 6. PubMed PMID: 23223333; PubMed Central PMCID: PMC3582066.

6: Young B, Eggenberger E, Kaufman D. Current electrophysiology in ophthalmology: a review. *Curr Opin Ophthalmol*. 2012 Nov;23(6):497-505. doi: 10.1097/ICU.0b013e328359045e. Review. PubMed PMID: 23047167.

**Tasks 2 and 3. In normal eyes, as well as in eyes with damage to the retina or optic nerve, collect, correlate, and define the normative range of values for both objective and standardized tests**

In Year 2 we submitted the forms and protocol to our local IRB3 Human Use Committee and finally did receive the IRB-approval, and this was followed by final approval by Brigit Ciccarello after submission to ORP HRPO after their second-level review and approval was received.

During Year 4, we have started testing normal subjects and patients to optimize our stimulus protocol for reducing inter-subject variability and maximizing the detection of disease. We have collected data from 2 normal subjects, as well as from 2 patients with unilateral optic nerve damage caused in one case by a pituitary tumor compressing one



side of the chiasm and in one case from anterior ischemic optic neuropathy (AION). Subjects were tested using the same protocols and data was collected for:

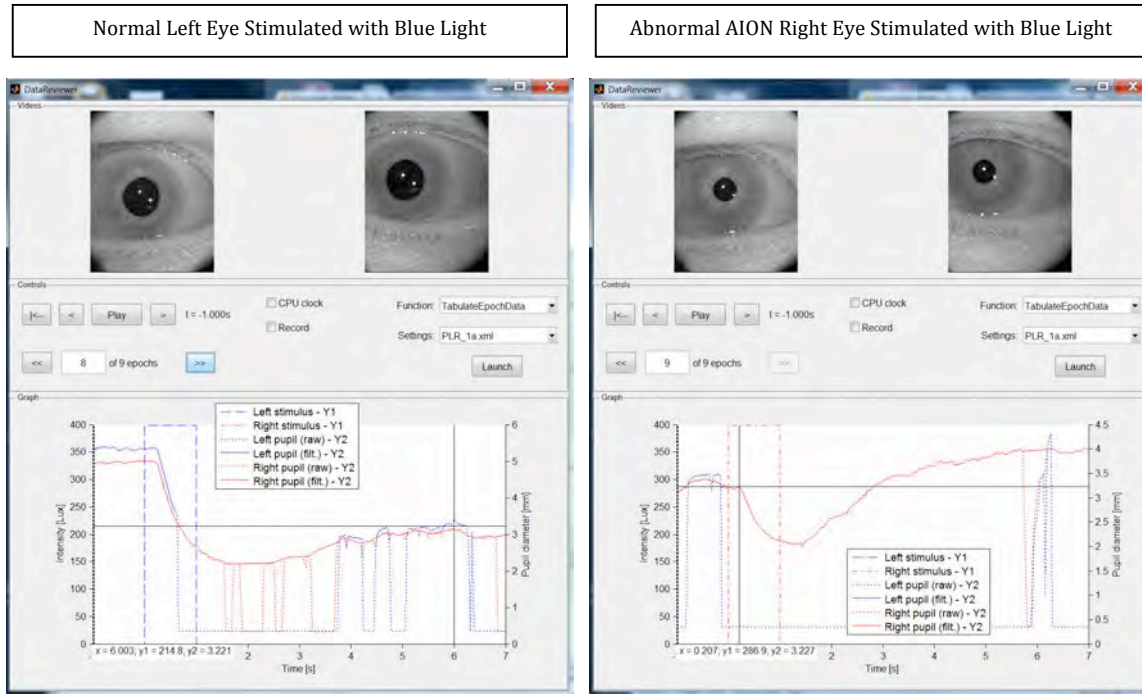
- Pupil light reflexes
- Evoked potentials
- Eye movements to targets changing in resolution
- Standard light threshold perimetry

We have analyzed these results to troubleshoot for any unanticipated problems. We also started the process of comparing the outcomes of our proposed objective tests to the results from standardized clinical tests, as well as evaluating different metrics from the objective tests for their diagnostic value in differentiating between normal and diseased states.

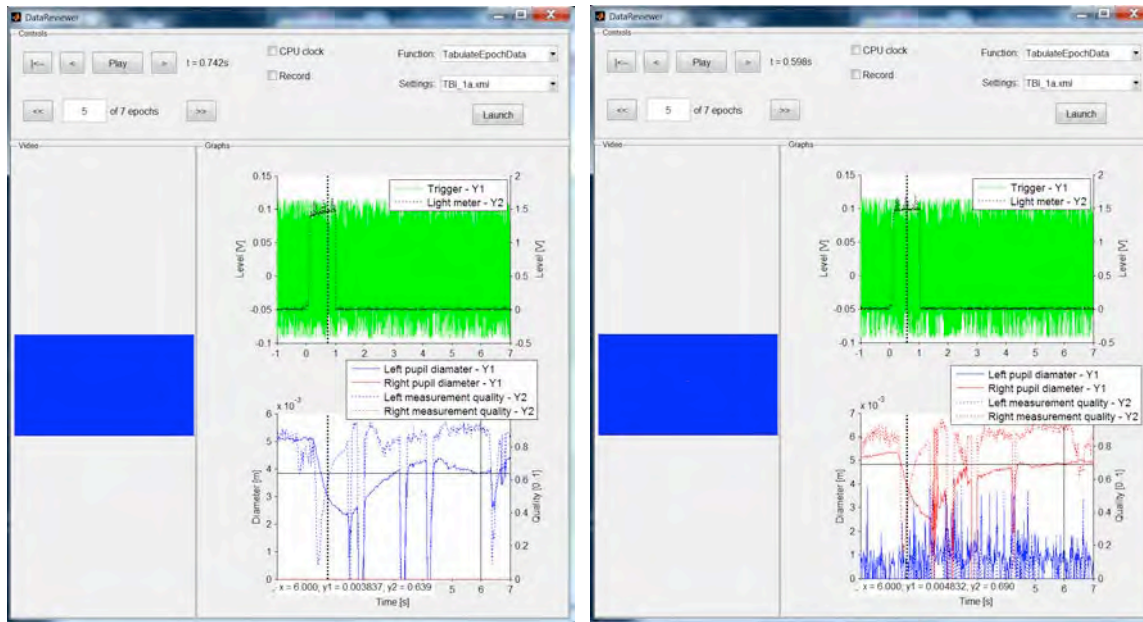
During the analysis process, we adapted the software tools we have developed as part of our other DOD/TATRC research project, titled "Use of the Photo-Electromyogram to Objectively Diagnose and Monitor Treatment of Post-TBI Light Sensitivity" to expedite data analysis and interpretation of results. We have reported elsewhere that we had implemented a Matlab GUI-enabled tool for reviewing and visualizing video and time series data within a unified and user-specified framework, simply by specifying the relevant input data sources and GUI layout parameters for each type of data recording session. Video and time series data are synchronized based on timestamps. The GUI enables the user to play back, rewind, etc. recorded data, and easily navigate to any time slice by clicking in any of the linked graphs, as well as jump to any specific stimulus epoch. The video frame that is displayed for each video stream is calculated on the fly and based on the current timestamp. The user has the ability to specify the number of graphs, as well as how each time series is to be assigned to any Y axis (both left and right for each graph), and can use the mouse-controlled crosshair to read off the x, y1 (left), and y2 (right) coordinates on any graph. The user can also record the GUI window in order to create movie clips for presentations, etc.

For the purposes of this report, we would like to describe the results for two tests, namely the red-blue pupil light reflex test, as well as the evoked potential test. Based on the Matlab GUI-enabled tool described above, Figure 7 and Figure 8 show examples of video and pupil data collected during the red-blue pupil light reflex test from the patient with AION with the Neuroptics DP-2000 system and our custom DynaScan-Smart Eye system. During this test, a series of 1s red and blue stimuli of varying intensities are presented to each eye separately or to both eyes simultaneously. As mentioned earlier in this report, we have developed a novel method to objectively diagnose the presence and severity of optic neuropathy within an individual eye independently, without having to reference it to the other eye as an asymmetry index. For this method, the % pupil contraction to a bright red stimulus is compared to the % pupil contraction to a bright blue stimulus as sustained 6s after stimulus onset. Comparison of the red vs. blue pupil response within each eye minimizes the impact of central nervous system influences on measurements of visual function and pupil response that are unrelated to the condition affecting vision. Such central nervous system influences having to do with state of excitement or sleepiness can

cause fluctuations in the response of the pupil unrelated to the eye's function and can confound the diagnosis of ocular disorders. Results for the patient with AION are tabulated in Table 3 for both the Neuroptics DP-2000 system and our custom DynaScan-Smart Eye system, and lend support to develop and evaluate the objective test further.



**Figure 7. Screenshots of the Matlab GUI tool populated with data collected from the Neuroptics DP-2000 system for the patient with AION in the right eye in response to two stimuli. On the left hand side, pupil responses for a 1s bright blue stimulus delivered to the left (healthy) eye are shown. Pupil responses for a 1s bright blue stimulus delivered to the right (diseased) eye are shown on the right hand side. In this example, data are displayed for  $t = -1$ s before stimulus onset (as indicated by the vertical black dotted line in each graph), while each pair of video frames is showing the eyes at this time instance (right eye on the right, and left eye on the left). Note that the blue stimulus for the right eye is delivered only 15s after the blue stimulus has been delivered to the left eye. This short inter-stimulus interval doesn't allow the pupils to recover sufficiently to dark-adaptation levels, which leads to reduced % contraction values for the right eye stimulus if not corrected, and can confound interpretation of the results given the fact that the right eye is also the diseased eye (AION). We will look into lengthening of the inter-stimulus interval between the bright blue stimuli, and evaluate the effects of either correcting for the significant pupil baseline discrepancy or instead using the % dilation from the minimum pupil size. Also note that the pupil tracking algorithm failed to locate the pupil border of the left eye for most of the time.**



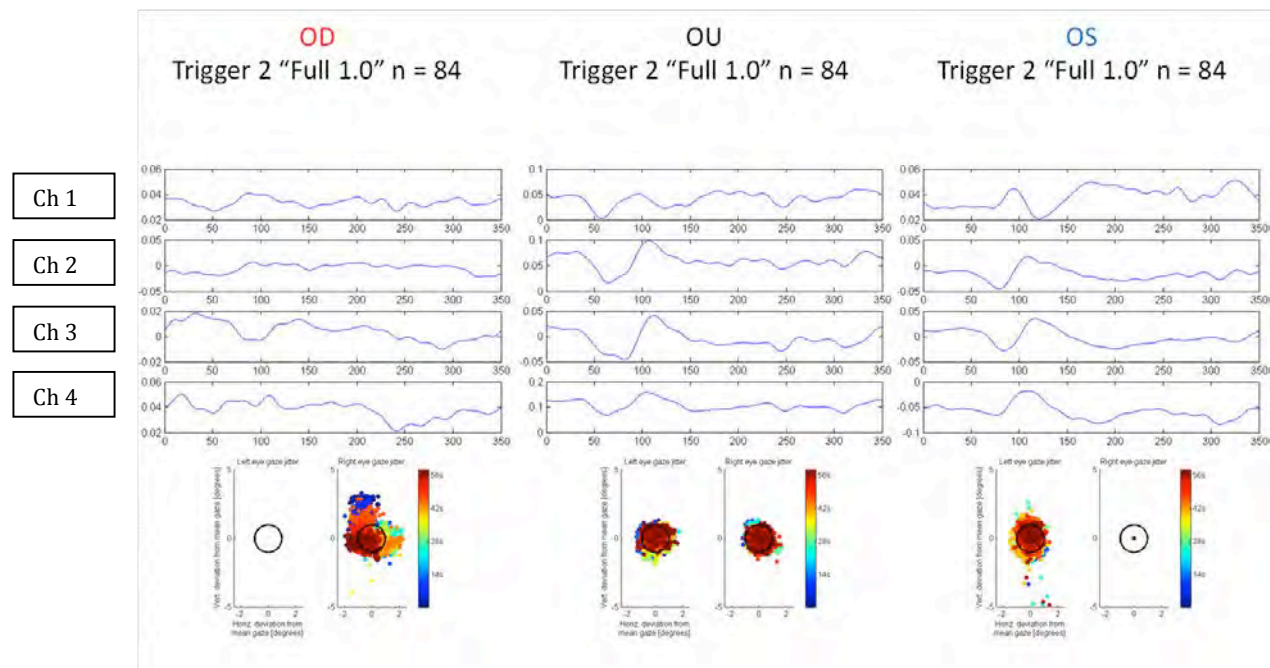
**Figure 8. Screenshots of the Matlab GUI tool populated with data collected from our custom-built DynaScan-Smart Eye system for the patient with AION in the right eye in response to two stimuli. On the left hand side, pupil responses for a 1s bright blue stimulus delivered to the left (healthy) eye are shown. Pupil responses for a 1s bright blue stimulus delivered to the right (diseased) eye are shown on the right hand side. In this example, data are displayed for instances of time during the 1s stimuli (as indicated by the vertical black dotted line in each graph), while the video frames show the screen contents displayed to the subject (in this case, a blue screen). After upgrading the Smart Eye head and eye tracking system (discussed later in the progress report), we will be able to add video frames of the subject's head and eyes to the GUI. Note that stimuli are not alternated between eyes in case of the DynaScan-Smart Eye system because the subject has to wear a filter in front of the eye not being tested to block light from the TV, as described in the previous progress report. Each eye is therefore tested independently. Also note that the sustained response to a bright blue stimulus delivered by the DynaScan TV is reduced compared to the response observed with the Neuroptics DP-2000 system probably because of the broad spectrum of the blue light emitted by the TV (as measured and described in the previous progress report), as well as the fact that we have not been able to keep the testing environment with the TV as dark as is possible during testing with the Neuroptics DP-2000 system.**

**Table 3. Results for the red-blue pupil light reflex test from the patient with AION with the Neuroptics DP-2000 system and our custom DynaScan-Smart Eye system. For both test platforms, the % pupil contraction value sustained at 6s after a bright blue stimulus is reduced for the diseased right eye (OD) in comparison to the healthy left eye (OS), while the pupil response to the bright red stimulus has dissipated by 6s. Note that the baseline pupil size for the right eye (OD) blue stimulus from the Neuroptics DP-2000 system has been adjusted to match the dark adaptation baseline**

for the right eye (OD) red stimulus, and the derived contraction values are marked with an asterisk.

Stimulus	Neuroptics DP-2000						DynaScan-Smart Eye					
	baseline (mm)	min. (mm)	@ 6s (mm)	% max. contraction	% contraction @ 6s	% dilation @ 6s	baseline (mm)	min. (mm)	@ 6s (mm)	% max. contraction	% contraction @ 6s	% dilation @ 6s
OS - red	4.8	2.5	4.6	48%	4%	84%	4.9	2.3	4.8	53%	2%	109%
OS - blue	5.2	2.2	3.2	58%	38%	45%	5.1	2.3	3.8	55%	25%	65%
OD - red	5.0	2.6	4.8	48%	4%	85%	5.2	3.0	5.2	42%	0%	73%
OD - blue	5.0* (3.4)	2.1	4.0	58%*	20%*	90%	5.3	2.4	4.8	55%	9%	100%
OU - red	4.9	2.4	4.7	51%	4%	96%	4.9	2.3	4.7	54%	3%	109%
OU - blue	4.9	2.1	2.8	57%	43%	33%	5.1	2.3	4.3	55%	16%	89%

In addition to the red-blue pupil light reflex test, we have also collected evoked potential data for the AION patient with the DynaScan-Smart Eye system. Overall, VEP responses are significantly attenuated when the diseased right eye is stimulated when compared against the VEP responses from stimulation of the healthy left eye or of both eyes simultaneously. Results are shown in Figure 9.

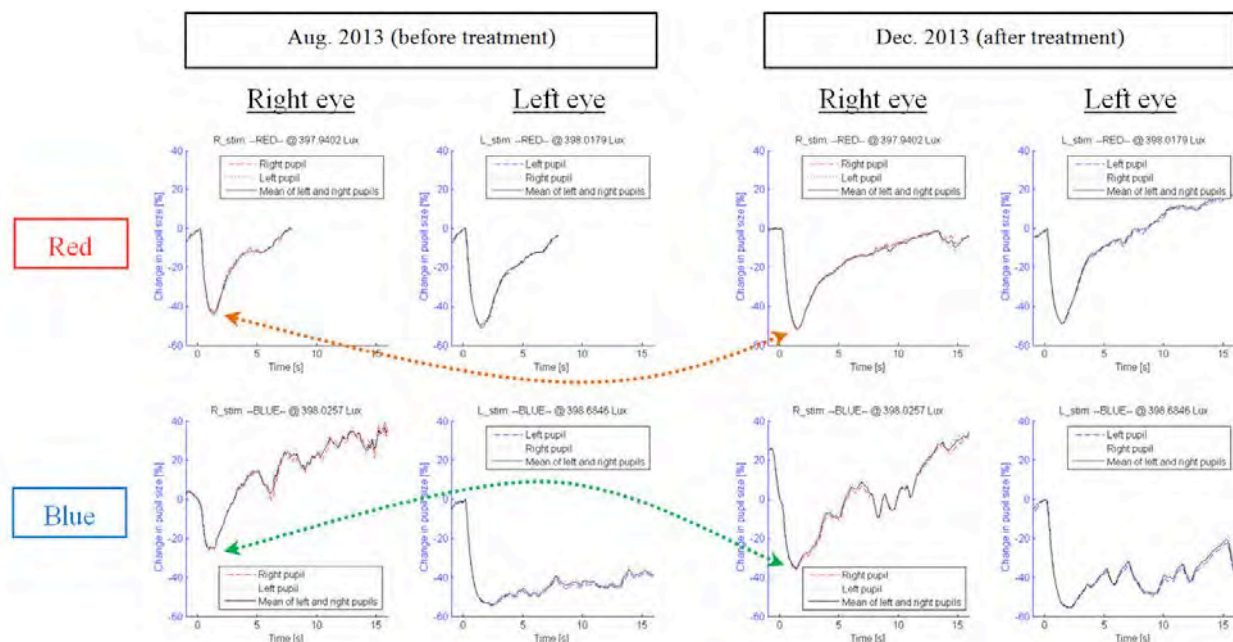


**Figure 9. VEP response functions for 4 EEG channels in response to full-field 1° checkerboard pattern onset/pattern offset stimulation for 3 testing conditions: right eye (OD) stimulation on the left hand side, both eye (OU) in the center, and left eye stimulation (OS) on the right hand side. The C1 (@ 80ms) and C2 (@ 110ms) peaks are clearly visible in channels 2 and 3 for the OU and OS test conditions, but attenuated for the OD condition. Gaze offsets are shown below each set of VEP graphs, colored coded according to the time offset of each gaze point with respect to**

**the beginning of the test, which lasts for about 60s. The black circle on each gaze jitter graph represents 1° offset with respect to the mean gaze vector during the test. Note that the patient has greater difficulty to stay fixated on the fixation target during stimulation of the (diseased) right eye (OD condition).**

We have earlier described the finding that the melanopsin mediated pupil response, derived from the differential sustained pupil response to blue light vs. red light, correlates highly with retinal ganglion cell layer thickness derived from optical coherence tomography (OCT) in eyes with optic neuropathy due to multiple sclerosis. We hypothesize that it will pave the way for determination of afferent input of each eye independent of the other and will be especially useful in patients with bilateral disorders of visual function, in which there may not be a relative afferent pupil defect. During the past year, we have further evaluated the diagnostic implications of this simple pupil light reflex test, as shown in Figure 10. This example is from a patient with right eye visual loss and optic nerve damage from an optic nerve sheath meningioma compressing the right optic nerve. The left eye was normal. In Figure 10, pupil tracings for red and blue stimuli, matched for photopic intensity, are shown for right and left eye stimuli, while recording both pupils. A deficit in both the initial pupil contraction to red and blue stimuli is seen with the right eye stimulus. In addition, the sustained pupil contraction, normally seen after a 1-second bright blue light stimulus, was not present, indicating damage to the retinal ganglion cells containing melanopsin. The patient was treated with radiation therapy and 4 months later returned with much improved vision and visual field. The patient's pupil contractions were also significantly greater, for both the initial contraction to red and blue light and she also showed an increase in the sustained pupil contraction to the blue stimulus. This example shows the utility of the initial pupil contraction to red and blue light stimulus for detecting disease in each eye and the utility of assessing the sustained pupil contraction to the blue light stimulus with respect to the red light stimulus. In addition we show here the utility of the pupil responses to red and blue light for monitoring treatment, which will have a significant clinical application.





**Figure 10. Raw pupil size tracings in response to individual stimulation of each eye with red and blue 1 second duration bright light stimuli before and after treatment. Each graph includes the tracings of the left pupil, right pupil, and the mean of the left and right pupil for the different eye and stimulus color combinations. Note the increase in the initial pupil response to both red light (orange arrow), as well as blue light (green arrow) following treatment.**

We have added Robert Mallery MD to our team for the next year as a research fellow to test normal subjects and patients with the eye movement/visual assessment platform developed for this DOD project. Dr. Mallery is a board certified neurologist and also recently completed a clinical neuro-ophthalmology fellowship. The addition of Dr. Mallery will accelerate our progress and subject recruitment in the coming year.

### **Task 5. Optimize hardware systems and vision testing protocols to reduce testing time, maximize the signal/noise, and minimize cognitive demands placed on the patient for proposed objective tests**

During the past year, an upgraded 120 Hz digital 4-camera head and eye tracking system from Smart Eye has been delivered to us. One of Smart Eye's image analysis experts visited our lab for a 2-day on-site visit to install and provide training for the new system, as well as assisted us to optimize the positioning of the 4 IR cameras around the DynaScan TV. Given that the Smart Eye system allows one to arrange the IR cameras in different configurations, we want to ensure that the system is optimized to track large rotations of a subject's head (not sideways head movements) while still tracking both eyes and pupils robustly. As mentioned in previous progress reports, the new system contains the following upgrades:

- At a 120 Hz, the sampling rate will be double the sampling rate of our current head and eye tracking system, which will enable denser sampling of saccadic eye movements as well as eyelid dynamics.
- The system will be able to record the raw, uncompressed video streams from the 4 cameras to disk at 120 Hz, while simultaneously tracking head and eye movements at 120 Hz. This capability will enable us to both make use of the head/eye tracking data for experiment control and monitor the quality of the tracking data in real time while the raw video data are also saved at the same time to allow additional image analysis afterwards.
- The time consuming task of building and registering a head model for each subject has been fully automated. The experimenter simply has to press a button to activate the system's tracking mode, which instructs the software to start the process of constructing a head model automatically, and fine-tuning the model over time. This capability is especially useful in case of young and/or uncooperative patients. Knowing both the head and eye position simultaneously in real time will allow unprecedented control of visual stimuli and analysis of responses.
- Previously, in order to estimate the optical axis of an eye, the software algorithm required that the eye had to be visible in at least two camera images. However, the latest version of the software makes better use of head and facial features to locate the head and eyes in 3D, which relaxes the requirement for any eye to be visible only in a single camera view, allowing us to increase the distance between neighboring cameras and therefore enabling us to track an even bigger range of head and eye orientations than possible with our existing system.

## **KEY RESEARCH ACCOMPLISHMENTS (SUMMARY)**

- Based on the encouraging results from the continuous RAPD testing and analysis protocol developed as part of this project, we have submitted an Invention Disclosure to the University of Iowa Research Foundation, titled "Objective Methods of Testing Visual Function Using the Pupil Light Reflex", with an eye on patenting the concept.
- We have recently determined that the melanopsin mediated pupil response, derived from the differential sustained pupil response to blue light vs. red light, correlates highly with retinal ganglion cell layer thickness derived from optical coherence tomography (OCT) in eyes with optic neuropathy due to multiple sclerosis. Based on our initial findings, we have submitted an Invention Disclosure to the University of Iowa Research Foundation, titled "Use of the Pupil Light Reflex to Chromatic Stimuli to Track Optic Neuropathy", with an eye on patenting the concept.
- In terms of the red-blue pupil light reflex test, we have shown that the resulting responses measured with our custom DynaScan-Smart Eye system correlate well with results obtained with the Neuroptics DP-2000 system. Specifically, for both test platforms, the % pupil contraction value sustained at 6s after a bright blue stimulus is reduced for the diseased eyes in comparison to the healthy eyes, while the pupil response to the bright red stimulus has dissipated by 6s.

- By comparing the pupil light reflex before and after radiation treatment of a patient suffering nerve sheath meningioma compressing the optic nerve, we were able to show the utility of the pupil responses to red and blue light for monitoring treatment. The recovery of the pupil light reflex in the diseased eye following treatment correlated with improvement in visual acuity, as measured with a standard clinical test.
- In terms of evoked responses, we have shown that VEP amplitudes in response to our design matrix stimulation method presented on our custom DynaScan-Smart Eye system are significantly attenuated when a diseased eye is stimulated when compared against the VEP responses from stimulation of the healthy eye or of both eyes simultaneously.
- We have found that a patient has greater difficulty to stay fixated on a fixation target during stimulation of a diseased eye.

## REPORTABLE OUTCOMES

1: **Kardon R, Poolman P**, Pienta JN, Full J, Anderson SC. A New Automated 20 Second Pupillographic Test to Quantify the Log Unit Relative Afferent Pupillary Defect and its Relationship to Retinal Ganglion Cell Thickness. ARVO Annual Meeting, Orlando FL, 2014.

2: **Poolman P**, Pienta JN, Full J, Anderson SC, **Kardon R**. The Influence of Eyelid Position and the Photic Blink Reflex Upon the Pupil Light Reflex. ARVO Annual Meeting, Orlando FL, 2014.

## CONCLUSIONS

The research work that we are carrying out has important implications for the greater public good, in addition to its military relevance. Visual impairment from traumatic brain injury can occur in military personnel exposed to direct trauma to the brain or indirectly from blast injury. Similar damage to the visual system can also occur in the civilian population from TBI resulting from motor vehicle accidents and also from head injury due to contact sports at both the school and professional level. Traumatic causes of visual damage can also be additive after repeated episodes of head injury. Patients with visual pathway damage are often unaware of the problem and their associated cognitive impairment may mask the underlying vision impairment and also prevent detection with standard tests of visual function, which require good cognitive performance and focused attention during the test. In addition, other forms of cognitive impairment in the general population such as attention deficit disorder, depression, and dementia prevent the accurate assessment of visual function. Patients with undiagnosed visual dysfunction and superimposed cognitive impairment may pose a danger to themselves and to others when tasks such as driving and other tasks, which demand good visual performance, cannot be safely carried out.

For this research, our main goal is to use objective reflexes of the visual system to diagnose vision deficits and ensure effective monitoring of their treatment, when indicated. Such tests will allow accurate testing of the visual system with almost no demands on cognitive



function during testing. This will be possible because the constriction of the pupils in response to light, the electrical recording of light evoked potentials (voltage) from the skin overlying the vision centers of the brain and the monitoring of purposeful eye movements to track moving targets are all objective, natural reflexes of the visual system. We are taking advantage of these reflexes by implementing an integrated system to quantify them using a specially designed suite of rapidly performed tests requiring little patient cooperation. Once validated in our proposed study, these tests can be used in cognitively intact or cognitively impaired individuals to assess visual function, leading to rehabilitation and treatment when appropriate.

The availability of the objective tests of vision being developed and implemented will greatly improve eye care by providing faster, lower cost testing that can be performed in remote settings. This will provide easier access of the public to accurate assessment of their visual function and will also reduce the cost associated with current testing and transportation to sites of testing. Such tests will also provide a new tool for assessing innovative treatments being developed to save or restore vision.

## **REFERENCES**

None.

## **APPENDICES**

None.

## **SUPPORTING DATA**

All figures including in body of report